Primary treatment of zygomycosis with liposomal amphotericin B: analysis of 28 cases

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Lipid formulations of amphotericin B are increasingly used in lieu of deoxycholate amphotericin B for primary treatment of zygomycosis, but little is known about the efficacy of the former antifungal in treating this fungal disease. We therefore undertook an analysis of a case series of all patients with zygomycosis who received L-AMB for primary antifungal therapy in five major mid-Atlantic medical centers. Among the categories of variables studied were demographics, methods of diagnosis, microbiology, sites of infection, global responses, and survival. The median patient age was 44 years and 71% were male. Immunosuppressive hematological disorders (54%) were the most common underlying condition. Pulmonary disease constituted 50% of infections, sinus infection 29%, and cutaneous disease 18%. Members of the genus Rhizopus were the most common recovered agents. Success as defined by complete or partial positive response was noted in 32% of the cases. Concomitant surgery was performed in 46% of the cases, with similar response rates (31%). Overall survival was 39%. L-AMB was effective as primary therapy in only some patients in this cohort of highly immunocompromised individuals with invasive zygomycosis underscoring the importance of host response and the need for further advances for treatment of this lethal infection.

Keywords: zygomycosis, liposomal amphotericin B, leukemia, hematopoietic stem cell transplantation, Rhizopus oryzae

Introduction

Zygomycosis is emerging as an uncommon, but potentially lethal, and increasingly important infection [1–5]. This increase has been especially apparent in hematopoietic stem cell transplant (H SCT) recipients and in patients with hematological malignancies [1,6–12], where crude mortality may exceed 60%. Zygomycosis also can be lethal in more immunocompetent hosts, such as those with diabetes mellitus, intravenous drug users and surgical patients [1,13–17].

To date, deoxycholate amphotericin B (DAMB) is the only antifungal agent approved by the US Food and Drug Administration (FDA) for primary treatment of zygomycosis. Since the mid-1990s, lipid formulations of amphotericin B (LFABs) have been used as salvage therapy for patients with zygomycosis. Given the more favorable safety profile of LFABs, these compounds are increasingly used for primary therapy. Retrospective reviews of the use of amphotericin B lipid complex (ABLC) and amphotericin B colloidal dispersion (ABCD) as primary and salvage treatment of zygomycosis have been reported [18–22].

Little is known, however, about the efficacy of liposomal amphotericin B (L-AMB) in the primary treatment of...
invasive zygomycosis, as no extensive review has been conducted of its effect in zygomycosis cases. We therefore reviewed the effect of L-AMB in the primary treatment of invasive zygomycosis within five medical centers located in one centralized mid-Atlantic region of the United States.

Methods

Study design

Institutional Review Board approval was obtained from all five centers participating in this study. These centers included The Warren Grant Magnuson Clinical Center of the National Institutes of Health, Georgetown University Medical Center, Children’s National Medical Center, The Johns Hopkins Hospital, and The Washington Hospital Center. Patients were identified by review of microbiology, pathology and clinical databases. The medical records of all patients with a diagnosis of invasive zygomycosis where L-AMB was used as the primary (initial and majority) therapy were reviewed. Demographic and clinical data were collected and entered into a database, as described below. Dosages and durations of therapy were ascertained from the medical records and from post-discharge data (when available).

Fungal identification

Specific fungal identification methods differed slightly among institutions. In general, specimens were inoculated onto Sabouraud dextrose agar (Emmons modification; SDA) plates and Inhibitory Mold Agar (IMA). Sub-cultures were prepared on potato flake agar (PFA) to enhance, as needed, fungal growth. Determination of the presence of zygomycetes was made by observation of growth rate, macroscopic morphology and color of the colonies followed by identification of characteristic microscopic morphology findings using lactophenol aniline blue scotch-tape preparation or MycoPerm (SDL, Des Plaines, IL) staining.

Definitions

Invasive disease. Patients were classified as having proven or probable disease as defined by EORTC/MSG criteria [23]. Patients whose specimens from normally sterile sites yielded a zygomycete or with biopsy specimens showing characteristic hyphal morphology with evidence for associated tissue damage were defined as having proven invasive disease. If the positive culture was obtained from either bronchoalveolar lavage (BAL) or sputum in an immunocompromised host, the patient was classified as having probable invasive disease providing that there was compatible radiology and/or symptoms. Patients whose sinus biopsies demonstrated deep sub-mucosal invasion or skin specimens showed deep dermal or subcutaneous invasion by hyphae characteristic of a zygomycete were also defined as having proven invasive zygomycosis.

Global response. Patients were categorized as having complete response, partial response, stable disease, or failure at the end of therapy.

Outcome. Mortality was assessed as either zygomycosis-specific or crude mortality. If the patient had evidence of zygomycosis on autopsy then it was stated that the mortality was due to the fungal infection. If no autopsy was performed, but zygomycosis was listed as a primary or secondary cause of death in the medical record, then the mortality was also attributed to zygomycosis. Unless otherwise stated, zygomycosis-specific attributable mortality is used throughout the remainder of this report.

Database development

Filemaker Pro software, version 5.5 (Filemaker inc.; Santa Clara, CA) was used to develop a database of categorical and continuous variables. The categorical variables included gender, ethnicity, primary underlying condition, neutropenic status, presence or absence of corticosteroids, organism, diagnostic method used for recovery of organism, site from which the organism was recovered, premortem or postmortem diagnosis, use of surgery, immunomodulation, hyperbaric oxygen, global response and outcome. The continuous variables included year of diagnosis, age of patient, and dose and duration of antifungal therapy.

Statistical analyses

Data were analyzed by Prism-Instat software (GraphPad Software, Inc. La Jolla, CA) for descriptive statistics of central tendencies and dispersion within the variables identified.

Results

There were 32 patients from five major medical centers who were treated with L-AMB for invasive zygomycosis over a seven-year period from September 1998 to August 2005, inclusively. Four of these patients were excluded from the analysis as they received L-AMB as salvage antifungal therapy. The results from this study focused on those newly diagnosed patients with invasive zygomycosis who received L-AMB as primary therapy.

Demographic characteristics

The mean patient age was 43 years and the median age was 44 years (range, 0.2–78; Table 1). A total of 71% of all zygomycete infections occurred in males. The overall mortality in patients receiving L-AMB as primary therapy for
zygomycosis was 61% (17 of 28 patients). Hematologic disorders including leukemia, bone marrow transplantation, aplastic anemia, myelodysplastic syndrome, and lymphoma were the most common underlying conditions and observed in 15 (54%) of 28 patients (Table 1). Pulmonary disease was the primary site of infection in 50% of patients, with other primary sites including sinus (29%), kidney (4%), and palate (4%) (Table 2).

Microbiological findings
All patients had proven or probable zygomycosis, as previously defined. Diagnosis was confirmed by the recovery of the etiologic agent in culture from specimens of 23 (82%) of 28 patients, with members of the genus Rhizopus being the most common isolates obtained in culture (Table 3).

Treatment and outcome
All patients received L-AMB for primary therapy of their infection. Dosages ranged from 3mg/kg to 14mg/kg. Based on the data available to us, the mean duration of therapy was 29 days in those patients who survived the infection. At the end of antifungal therapy, 9 (32%) of the 28 patients had either partial or complete positive responses. Three (11%) others were classified as having stable disease and the remaining 16 (57%) as having failed antifungal therapy. Statistically significant differences were not detected with regards to dosages of L-AMB and outcomes.

Overall mortality for patients treated with L-AMB was 61%, with the mean duration of therapy until death of 24 days (Figure 1). Outcomes by type of adjunctive therapy are shown in Table 4. Seventy-eight percent of those patients who received L-AMB alone survived. Surgery was performed with 13 patients (46%), of whom 9 (69%) died. Four of these patients had disseminated zygomycosis at baseline, with two dying within a week of diagnosis and one of disseminated aspergillosis. There were 10 additional patients who received concomitant immunomodulation through colony stimulating factors or

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (%) of all patients</th>
<th>Proportion (%) of patients who died</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>28</td>
<td>17 (61)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>43</td>
<td>---</td>
</tr>
<tr>
<td>Median (range)</td>
<td>44 (0.2–78)</td>
<td>---</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (71)</td>
<td>12/20 (60)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (29)</td>
<td>5/8 (63)</td>
</tr>
<tr>
<td>Underlying condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>6 (21)</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>Aplastic Anemia/MDS</td>
<td></td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>Solid Tumor</td>
<td>3 (11)</td>
<td>2/3 (67)</td>
</tr>
<tr>
<td>Primary Immunoodeficiency</td>
<td></td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2 (7)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>Solid organ transplantation</td>
<td></td>
<td>1/2 (50)</td>
</tr>
<tr>
<td>Bone marrow transplantation</td>
<td></td>
<td>1/2 (50)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (7)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>Surgery</td>
<td>1 (4)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td></td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>Intranasal drug use</td>
<td></td>
<td>0/1 (0)</td>
</tr>
</tbody>
</table>

aData are expressed as number and proportion (%) of patients, unless otherwise specified.

bData are expressed as number of patients with the characteristic who died/total number with the characteristic (%).
L-AMB as primary therapy for zygomycosis was 61% (17 of 28 patients) and a response at the end of therapy was seen in 32% (9 of 28 patients).

Invasive zygomycosis is an uncommon but frequently fatal infection. Outcomes are dependent upon multiple variables including timely initiation of effective antifungal chemotherapy, surgical factors, host immune status, site and extent of infection at time of presentation and use of adjunctive immune modulating therapy. Antifungal chemotherapy and surgery, particularly in extra-pulmonary infections, remains the mainstay of treatment for zygomycosis. Infection is nearly uniformly fatal in the absence of these modalities [1]. DAMB currently is the only FDA approved agent for primary treatment of zygomycosis. The limitations of this compound in terms of infusion granulocyte transfusions. The overall survival was 30% in this subgroup.

**Discussion**

In this study, we report 28 patients with invasive zygomycosis who were treated with liposomal amphotericin B as primary therapy, the largest such investigation in the literature. The most common underlying conditions were hematological disorders followed by solid tumors, inherited immunodeficiencies, transplantation and diabetes mellitus. The most common site of infection was the lungs, followed by sinuses and skin. The most commonly recovered genus was *Rhizopus* followed by *Mucor* and *Rhizomucor*. The overall mortality in patients receiving L-AMB as primary therapy for zygomycosis was 61% (17 of 28 patients) and a response at the end of therapy was seen in 32% (9 of 28 patients).

Table 4 Treatment administered to 28 patients with zygomycosis who received primary L-AMB therapy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number (%) of all patients</th>
<th>Number (%) of all patients with a successful response*</th>
<th>Number of patients who survived/total number who received treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-AMB for primary therapy</td>
<td>28 (100)</td>
<td>9/28 (32)</td>
<td>11/28 (39)</td>
</tr>
<tr>
<td>Concomitant surgery</td>
<td>13 (46)</td>
<td>4/13 (31)</td>
<td>4/13 (31)</td>
</tr>
<tr>
<td>Concomitant immunomodulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>10 (36)</td>
<td>2/10 (20)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>GCSF</td>
<td>7 (25)</td>
<td>2/7 (29)</td>
<td>3/7 (43)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1 (4)</td>
<td>0/1 (0)</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>Granulocyte transfusions</td>
<td>2 (7)</td>
<td>0/1 (0)</td>
<td>0/2 (0)</td>
</tr>
</tbody>
</table>

*Successful response is defined as complete or partial global response at end of L-AMB therapy.
related toxicity and dose limiting nephrotoxicity are well described [24–26].

The advent of LFABs has allowed for higher doses of amphotericin B to be delivered with substantially less toxicity. Daily L-AMB dosages in this study ranged from 3 mg/kg to 14 mg/kg and are substantially higher than would have been possible with DAMB. Whether such dosages of L-AMB are associated with improved outcomes in treatment of zygomycosis is uncertain. According to the data available to us, the median duration of therapy was 29 days in patients that survived. However, complete details of outpatient therapy were unavailable leading us to underestimate the length of therapy in some patients. There was no pattern of improved response of zygomycosis in relation to dosage of the drug in this study. Moreover, an earlier study of the safety and pharmacokinetics of L-AMB also found no correlation between patient outcomes and dosages extending from 7.5 mg/kg/day to 15 mg/kg/day in treatment of different fungal pneumonias in neutropenic patients [27]. In that study, serum drug levels reached maximum values following administration of 10 mg/kg/day and declined at higher doses suggesting a possible change in the elimination mechanism at high amphotericin B concentrations. The investigation of Cornely et al. of L-AMB in treatment of invasive aspergillosis in patients with leukemia also found no advantage in the use of a 10 mg/kg/day dosage in comparison to 3 mg/kg/day [28]. While the optimal dosage for treatment of zygomycosis is not defined, 5–7.5 mg/kg is a widely used dose in clinical practice [29].

Thus, dosages exceeding the range of 5.0–7.5 mg/kg/day, which were most commonly used in patients of this study, would have been unlikely to have improved outcomes.

Patients with zygomycosis often require a multifaceted approach to therapy including antifungal therapy, surgical resection when feasible, and reversal of immune impairment [30]. In this study surgery was performed in 46% of patients and immune modulation with growth factors and granulocyte transfusions were employed in 36% of patients. Despite these adjunctive modalities, mortality was high (~70% for both groups), underscoring the devastating potential of zygomycosis in the context of highly immunocompromised patients with multiple co-morbid conditions. While surgery may have a more definitive role in patients with sino-orbital and rhinocerebral zygomycosis in patients with diabetes mellitus [1,31], the role of surgery in patients with extensive pulmonary zygomycosis and hematological malignancies is not as well defined. However, interpreting differences in outcomes between surgical and non-surgical patients with zygomycosis is difficult given the multiplicity of factors affecting suitability for surgery in such patients and their relationship to overall survival.

In this review, outcomes varied as a function of the extent of disease. Among patients with respiratory tract zygomycosis, those with limited pulmonary disease tended to have the lowest mortality (36%). By comparison, extensive pulmonary, cutaneous, and disseminated zygomycosis were associated with 100% mortality. These differences are likely due, in part to differences in burden of infection and immune status of the patients. The pharmacokinetic properties of L-AMB may vary among tissue sites. For example, L-AMB in rabbits penetrates well into the lungs, particularly into pulmonary epithelial lining fluid [32]. However, the response of pulmonary zygomycosis to liposomal amphotericin B also is dependent upon innate host defenses. Previous in vitro studies demonstrate that GM-CSF augments human polymorphonuclear leukocyte-induced hyphal damage in response to Rhizopus oryzae [33]. Consistent with these findings, recent laboratory animal studies of experimental disseminated zygomycosis demonstrate improvement in survival in mice treated with L-AMB plus GM-CSF in comparison to those treated with single agents [34].

Laboratory animal studies and preliminary clinical observations suggest improved outcome with L-AMB in combination with echinocandin [29,31,35]. An echinocandin was not used as concomitant antifungal therapy among the patients in this investigation. Patients also did not receive posaconazole either as salvage or as combination therapy with L-AMB.

The overall survival and response rates in this study are consistent with a highly immunocompromised patient population. Roden et al. reviewed published reports of 929 patients with zygomycosis, 22% of whom had an immunosuppressive hematological condition or HSCT and 36% had diabetes mellitus. Reflecting the importance of host response, 34% of patients with malignancies and HSCT survived, but 56% of those with diabetes mellitus survived. Indeed diabetes mellitus was an independent variable that predicted survival in multivariate analysis, perhaps as a reflection of the reversibility of host impairment [1]. The recently reported survival of 100% (6/6) of patients receiving L-AMB for diabetes associated zygomycosis is consistent with this concept of the importance of the host response [31]. By comparison, the relatively high mortality observed in our patients is consistent with a population with severe, and difficult to reverse, immune dysfunction. In the present study 54% of patients had an underlying immunosuppressive hematological condition or hematopoietic stem cell transplantation. The response rates observed here are lower than those seen in patients previously treated on salvage therapy with other lipid formulations. These disparities may be a reflection of differences in host response and are in line with the 64% mortality reported for a cohort of 18 HSCT recipients with zygomycosis, the majority of whom were treated with LFAB [35].

Other LFABs have been used for primary or secondary treatment of zygomycosis. The initial study of salvage
therapy with ABLC for patients with zygomycosis who were intolerant of or refractory to conventional amphotericin B found a complete or partial response rate of 71% among 17 of 24 cases [22]. Larkin et al. reported their observations of ABLC in the treatment of 64 patients with zygomycosis in which they noted response rates at 72% (80% response for primary therapy and 69% for salvage) [18]. However, in that study stable disease was considered as a successful response and there were considerably more patients with diabetes mellitus (28%). Herbrecht et al. described their experience with ABLD as salvage treatment of zygomycosis [20]. Of the 20 patients in their review, 60% were successfully treated as defined by cure or improvement in their zygomycosis. Of these patients, 60% had hematological malignancy and 25% had diabetes as their primary underlying conditions. Gleisner et al. reviewed published reports of L-AMB treatment of zygomycosis in patients with underlying hematological or oncological disorders [37] and found survival of 62.5% (10/16) of the patients. However, comparison of outcomes between studies is difficult given the small numbers of patients and major differences in study design.

In conclusion, L-AMB had activity as primary therapy in some patients in a cohort of highly immunocompromised patients with zygomycosis, but overall outcomes remained poor, underscoring the importance of host response and the need for further advances for treatment of this lethal infection.

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References


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